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Effects of short-term growth hormone treatment on liver and muscle transcriptomes in rainbow trout (*Oncorhynchus mykiss*)

Scott A. Gahr,[1] Roger L. Vallejo,[1] Gregory M. Weber,[1] Brian S. Shepherd,[2] Jeffrey T. Silverstein,[1] and Caird E. Rexroad III[1]

[1]*National Center for Cool and Cold Water Aquaculture, Agriculture Research Service, United States Department of Agriculture, Kearneysville, West Virginia; and* [2]*Great Lakes WATER Institute, Agriculture Research Service, United States Department of Agriculture, Milwaukee, Wisconsin*

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Gahr SA, Vallejo RL, Weber GM, Shepherd BS, Silverstein JT, Rexroad CE 3rd. Effects of short-term growth hormone treatment on liver and muscle transcriptomes in rainbow trout (*Oncorhynchus mykiss*). *Physiol Genomics* 32: 380–392, 2008. First published December 11, 2007; doi:10.1152/physiolgenomics.00142.2007.—Although studies have established that exogenous growth hormone (GH) treatment stimulates growth in fish, its effects on target tissue gene expression are not well characterized. We assessed the effects of Posilac (Monsanto, St. Louis, MO), a recombinant bovine GH, on tissue transcript levels in rainbow trout selected from two high-growth rate and two low-growth rate families. Transcript abundance was measured in liver and muscle with the Genome Research in Atlantic Salmon Project (GRASP) 16K cDNA microarray. A selection of the genes identified as altered by the microarray and transcripts for insulin-like growth factors, growth hormone receptors (GHRs), and myostatins were measured by real-time PCR in the liver, muscle, brain, kidney, intestine, stomach, gill, and heart. In general, transcripts identified as differentially regulated in the muscle on the microarray showed similar directional changes of expression in the other nonhepatic tissues. A total of 114 and 66 transcripts were identified by microarray as differentially expressed with GH treatment across growth rate for muscle and liver, respectively. The largest proportion of these transcripts represented novel transcripts, followed by immune and metabolism-related genes. We have identified a number of genes related to lipid metabolism, supporting a modulation in lipid metabolism following GH treatment. Most notable among the growth-axis genes measured by real-time PCR were increases in GHR1 and -2 transcripts in liver and muscle. Our results indicate that short-term GH treatment activates the immune system, shifts the metabolic sectors, and modulates growth-regulating genes.

microarray; insulin-like growth factor; myostatin

SOMATIC GROWTH IS DEFINED as an increase in body size or weight and is regulated by the interaction of external (environmental and nutritional) and endogenous (hormonal) stimuli. A fundamental role for growth hormone (GH; also known as somatotropin) in rainbow trout (*Oncorhynchus mykiss*) somatic growth has been established by hypophysectomy and GH replacement studies (6, 32). A "dual effector theory of action" has been proposed for GH's growth stimulatory activity (27, 30) in which GH acts through the growth hormone receptors (GHRs) and alters growth and metabolism either directly or via stimulation of insulin-like growth factor (IGF)-I secretion. GH induces IGF-I secretion by the liver to act systemically in an endocrine fashion or by other tissues to act in an autocrine or paracrine fashion (8).

Studies have shown that treatment with heterologous and homologous GHs dramatically increases somatic growth in fish (15, 47), as demonstrated in GH transgenic salmon, which grow 5–30 times larger than their nontransgenic siblings (14). In the rainbow trout, a 6-wk recombinant bovine somatotropin (rbST) treatment significantly increased weight and length (21). Determinations of the underlying biological pathways enhancing growth in the salmonids after recombinant bovine GH (rbGH) treatment have been limited to individual gene expression studies (3, 4, 5, 26) and measurements of metabolic parameters (21, 28, 29, 42, 34, 57). For example, studies showed that GH treatment increases in vitro liver fatty acid secretion (44), reduces the size of developing adipocytes in culture (17), and reduces the liver lipid content in vivo (57).

Myostatin (also known as growth/differentiation factor 8) is a member of the transforming growth factor (TGF)-β family and in mammals is a negative regulator of skeletal muscle growth (36). In rainbow trout three myostatin (MSTN) transcripts have been identified, and these transcripts show much broader expression patterns than in mammals (22, 23), suggesting a more diverse activity than in mammals. Although GH is known to modulate expression of the myostatin-1 transcripts after GH administration in the muscle (3), modulation of myostatin's expression outside the muscle has not been investigated.

Until recently, analysis of gene expression in the salmonids has been limited by the lack of genomic information to support tissue-level transcriptome analyses. The large-scale expressed sequence tag (EST) projects conducted for rainbow trout and Atlantic salmon (13, 63) have supported development of high-throughput platforms for transcriptome analysis. The Genome Research in Atlantic Salmon Project (GRASP) has produced a 16K salmonid cDNA microarray containing 13,421 Atlantic salmon and 2,576 rainbow trout cDNA features (63). This chip has been validated for use in Atlantic salmon, rainbow trout, chinook salmon, and rainbow smelt and has proven to be valid for the identification of differentially expressed genes in the rainbow trout under a variety of physiological conditions (31, 46, 51).

Microarray analysis of gene expression modulation following GH treatment may provide a wealth of information with respect to pathways regulated during growth and development in the fish. To date, only one paper using the GRASP 16K microarray to monitor the effects of exogenous GH on gene expression has been published (49). That study investigated the effects of food ration on hepatic gene expression in GH

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Address for reprint requests and other correspondence: S. A. Gahr, NCCCWA/ARS/USDA, 11861 Leetown Rd., Kearneysville, WV 25430 (e-mail: scott.gahr@ars.usda.gov).

transgenic coho salmon. The primary objective of the present study is to determine the differential short-term effects of GH administration on gene expression in the liver and muscle of rainbow trout selected because of high or low growth rate. In addition to using the microarray, we used real-time PCR to measure genes central to growth regulation (IGFs, GHRs, MSTNs) and select genes identified by microarray analysis on low-growth rate fish treated with Posilac. Finally, we examined gene expression of both previously identified growth-related genes and those identified with the microarray in eight different tissues with real-time PCR, to evaluate the tissue specificity of the responses observed in the liver and muscle.

MATERIALS AND METHODS

Animal handling. Rainbow trout (hatched March 2005) selected for growth rate were obtained from National Center for Cool and Cold Water Aquaculture (NCCCWA) stocks. Families were selected based on body weight at 4 mo of age and thermal growth coefficient for the final month of growth. The two high-growth families used in the study were in the top 2% in terms of growth rate, and the low-growth families were in the lowest 10% for growth rate. From each family, 10 fish were randomly selected and placed in a 100-liter flow-through tank at ~13°C and dissolved oxygen at >90% saturation, one tank for each family. The average weights of the fish selected from the high-growth rate families were 195.3 ± 31.4 g and 224.3 ± 21.6 g and those from the low-growth rate families were 144.0 ± 30.0 g and 159.1 ± 37.4 g. Throughout the experiment fish were fed once daily at 2% body weight with commercial trout food (55% protein and 15% fat; Zeigler Bros, Gardner, PA). All animal handling procedures were reviewed and approved by the NCCCWA animal care and use committee (NCCCWA Institutional Animal Care and Use Committee no. 031).

Experimental design and sample collection. Fish were acclimated to the new tanks for 2 wk before initiation of the treatments. Fish from each family were randomly selected to receive one of three treatments: *1*) Posilac injection (120 mg/kg body wt; $n = 4$/family), *2*) vehicle injection ($n = 4$/family), or *3*) untouched controls ($n = 2$/family). For this study, Posilac slow-release rbST was kindly provided by Dr. Gregg Bogosian (Monsanto, St. Louis, MO). For the injection, fish were anesthetized by submersion in 75 mg/l tricaine methanesulfonate (Western Chemical, Ferndale, WA). The fish were injected intraperitonally with 40–70 μl of Posilac just anterior to the pelvic fins with a 1-ml syringe and an 18-gauge needle. After injection, the caudal fin was notched to allow treatment identification during tissue collection, and the fish were returned to their tanks.

Three days after injection, fish were removed from the tanks and euthanized with an overdose of tricaine methanesulfonate (≥250 mg/l). A blood sample was collected from the caudal vasculature in a heparinized syringe, and plasma was isolated by centrifuging whole blood samples at 10,000 *g* for 10 min at 4°C. Plasma samples were stored at −80°C until IGF-I analysis. Liver, white muscle, kidney, heart, gill filament, stomach, intestine, and brain samples were collected immediately from each fish. All tissue samples were flash frozen in liquid nitrogen and stored at −80°C until RNA isolation.

Plasma IGF-I assay and statistical analysis. Plasma IGF-I concentration was measured in plasma of all fish in the study and was determined as previously described (56) with ^{125}I-labeled recombinant salmon IGF-I (GroPep, Thebarton, Australia) for label and standards and the anti-barramundi IGF-I primary antibody (GroPep). Inter- and intra-assay coefficients of variation were 5% and 4%, respectively. The detection limit of the assay, defined as the 90% binding, was 0.10 ± 0.01 ng/ml. Samples were measured in triplicate. The statistical significance of main factors (GH treatment and growth rate) and the GH treatment × growth rate interaction effects were assessed with SAS Proc GLM (SAS, Cary, NC) and least significant difference LSD post hoc test. For all measurements a P value of <0.05 was considered significant.

RNA isolation. Total RNA was isolated from all tissues after TRI Reagent (Sigma-Aldrich, St. Louis, MO) modification of the guanidinium thiocyanate-phenol-chloroform method (11). RNA was dissolved in 20–50 μl of nuclease-free water and subjected to DNase treatment according to the manufacturer's protocol (DNase RQ-1, Promega, Madison, WI) to remove genomic DNA contamination. The DNase treatment was followed with a reextraction with TRI Reagent to remove residual DNase activity. RNA quantities were measured with the NanoDrop ND-1000 UV-Vis Spectrophotometer (NanoDrop Technologies, Wilmington, DE). The quality of the RNA was assessed by agarose gel electrophoresis (all RNAs) or by the Experion Automated Electrophoresis System (for microarray RNAs; Bio-Rad Laboratories, Hercules, CA) for visualization of the 28S and 18S rRNA bands. All samples were found to have a 28S-to-18S ratio of >1.5. All RNA samples were stored at −80°C.

Microarray hybridization. A cDNA microarray representing 16,006 Atlantic salmon and rainbow trout EST sequences (63) was purchased from GRASP. The full list of features on this slide can be obtained at **http://web.uvic.ca/cbr/grasp/array.html**. This microarray chip has been validated (63) and shown useful for gene expression analysis in the rainbow trout (46, 51).

For hybridization, complementary DNA containing amino-modified bases was synthesized with Invitrogen's Superscript Indirect cDNA labeling system (Invitrogen, Carlsbad, CA) and labeled with the Alexa Fluor 555 or 647 dyes (Invitrogen) according to the manufacturer's protocol with minor modifications. Briefly, 15 μg of total RNA was reverse transcribed with anchored oligo(dT)s, dNTPs (including amino-modified dNTPs), and Superscript III RT (Invitrogen). The cDNA was then purified by S.N.A.P. column purification (Invitrogen) and concentrated with the Microcon YM-30 concentrators (Millipore, Billerica, MA). The cDNA was reconstituted in the coupling buffer and mixed with either Alexa Fluor 555 or 647 reactive dye (Invitrogen). After 1- to 2-h incubation for dye coupling, excess dye was removed by S.N.A.P column purification and the amount of dye incorporation was determined with the NanoDrop ND-1000 UV-Vis Spectrophotometer (NanoDrop Technologies). For each slide, 15 pmol of each labeled cDNA was combined, concentrated with the Microcon YM-30 concentrators (Millipore), and mixed with 75 μl of SlideHyb no. 2 (Ambion, Austin, TX) for hybridization.

Microarray slides were prepared for hybridization by washing twice in 0.2% SDS for 5 min each, rinsing in ultrapure water for 5 min, and finally rinsing in warmed ultrapure water for 3 min. Slides were dried by centrifugation and placed in the humidified InSlide Out hybridization oven (Boekel Scientific Feasterville, PA) at 60°C. The combined labeled samples in hybridization buffer were heated to 95°C for 10 min, and the warmed mixture was pipetted under a LifterSlip coverslip (Erie Scientific, Portsmouth, NH) on top of the slide. The slides were then hybridized at 50°C for 16–18 h in the InSlide Out hybridization oven.

After hybridization, slides were washed on an orbital shaker for 5 min each in 2× SSC + 0.5% SDS, then 0.5× SSC, and finally 0.05× SSC, all at room temperature. Slides were dried by centrifugation and immediately scanned with a ScanArray Express Microarray Scanner (Perkin-Elmer, Waltham, MA). All slides were scanned at 5-μm resolution with the photomultiplier tube gain adjusted for ~1% of the spots saturated in each channel.

Array experimental design. This study included a total of 16 two-channel arrays and was designed for direct comparison between the GH- and vehicle-injected groups (see Supplemental Table S3).[1] That is, for each of the four groups, *1*) high-growth rate liver, *2*) low-growth rate liver, *3*) high-growth rate muscle, and *4*) low-growth rate muscle, four slides were hybridized, using unique RNA

[1] The online version of this article contains supplemental material.

samples from different fish. RNA isolated from each tissue or organ sample was reverse transcribed separately (without pooling) so that individual slides represented a biological replicate. Two slides were hybridized with the cDNA from the GH-injected fish labeled with Alexa Fluor 555 and the cDNA from the vehicle-injected fish labeled with Alexa Fluor 647, and two slides, using unique RNA samples, were hybridized with the GH cDNA labeled with Alexa Fluor 647 and the vehicle cDNA labeled with Alexa Fluor 555 within each tissue and growth rate (Supplemental Table S3). Sixteen slides were used in the present study, representing 32 individual tissue samples, for a total of 4 biological replicates for each treatment group. We used plasma IGF-I levels as a positive marker of GH bioactivity. We used only the GH- and vehicle-injected samples for the microarray portion of this experiment because we did not detect a statistically significant difference in plasma IGF-I levels between the vehicle-injected and untreated control fish ($P = 0.48$).

Microarray spot finding. The images generated by the ScanArray Microarray Scanner (Perkin-Elmer) were processed and spots were quantified with ScanArray Express software (Perkin-Elmer). Data were collected by the adaptive circle method with background subtraction. The analysis was set to flag all spots with a signal intensity of <1,000 and signal-to-noise ratio of <3. Data were exported to Microsoft Excel (Microsoft, Redmond, WA) for further analysis.

Array data preprocessing. The raw data intensities collected from two-channel arrays were preprocessed with scripts written in R language. These scripts included functions from the statistical packages MARRAY, ARRAYQUALITY, and LIMMA from the Bioconductor project (25). The array data preprocessing included arrays diagnostics, data quality assessment, data filtering, data background correction, and data normalization as outlined elsewhere (24). The scripts for data preprocessing are available on request (**roger.vallejo@ars.usda.gov**).

Data filtering. The raw data intensities generated by the ScanArray Express software (Perkin-Elmer) had a column flag with values of 1 = spot not found, 3 = good spot, and 4 = bad spot. Spots with flags of 1 and 4 were filtered out, in addition to those with columns Name and ID that had these remarks: Name = "Empty," "Blank Well," "Empty Spot" and ID = "NAC."

Background correction. For background correction to assess differential expression, we used the method "normexp" with offset = 50 implemented in the computer package LIMMA, which was often found to be more robust than a simple background subtraction when using outputs from most image analysis programs (60). This method adjusts the foreground adaptively for the background intensities and results in strictly positive adjusted intensities. The use of this offset allows for the filtering out of gene expression data with signal intensities <50. The effect of using this background adjustment method is to stabilize the variability of the M values [i.e., $M = \log 2(\text{Cy5}) - \log 2(\text{Cy3})$] as a function of signal intensity.

Data normalization. A within-array or two-channel normalization method was used to perform direct comparisons of RNA samples hybridized in the same array. The "robust spline normalization" method of data normalization (61) was used.

Statistical analysis of microarray data. The statistical analysis of preprocessed microarray data was performed with scripts written in R language. These scripts included functions from the statistical package LIMMA (58) from the Bioconductor project (25). The scripts for statistical data analysis are available on request (**roger.vallejo@ars.usda.gov**).

The study design included three factors with two levels each: GH treatment, tissue, and growth rate. The focus of this study was to identify differentially expressed genes due to hormone treatment effect (growth hormone vs. vehicle). Therefore linear models were fitted for each of four treatment groups (e.g., muscle-high, muscle-low, liver-high, and liver-low) to identify genes that had significant differential gene expression for the "hormone treatment" effect. The "dye" effect was included in the fitted linear models because about one-third of the 17,328 tested features had significant differential gene expression (P value <0.01) for the "dye" effect.

The basic statistic used for significance analysis is the moderated t-statistic, which is computed for each probe and for each contrast. This has the same interpretation as an ordinary t-statistic except that the standard errors have been moderated across genes (i.e., shrunk toward a common value) with a simple Bayesian model (59). The adjusted P values (aka q values) are an estimate of the false discovery rate (FDR). We calculated the FDR by the Benjamini and Hochberg (2) method. For all hybridizations, all features with a P value of <0.01 and a FDR of <0.2 were considered to be significantly different, with an exception for the high-growth rate liver samples, in which the lowest FDR was 0.423.

Real-time PCR analysis. Real-time PCR analysis was conducted as previously described with minor modifications (23). Briefly, 2 μg of total RNA was reverse transcribed with random primers (Promega, Madison, WI) and Moloney murine leukemia virus reverse transcriptase (MMLV-RT, Promega) in a 40-μl reaction. Subsequent real-time RT-PCR assays were conducted with the ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA) and transcript-specific primers (Table 1 or previous publication; see below). The reaction was conducted with 1 μl of cDNA (1:5 dilution of RT reaction) combined with 5 μl of 2× SYBR Green PCR master mix (Applied Biosystems). For each reaction, the 6-μl mixture was added to 9 μl of primer mix containing an appropriate amount of each primer (see Table 1) and 2.5 μl of the 2× SYBR Green master mix (Applied Biosystems). The reactions were carried out as follows: 50°C for 2 min, 95°C for 10 min, and then 40 cycles consisting of 95°C for 15 s and the annealing temperature for 1 min (see Table 1). The cycling reactions were analyzed by a dissociation curve to verify amplification of a single product. The relative standard curve method was employed to quantify gene expression, as previously described (23). For each primer set, a serial dilution of a mixed-tissue cDNA was used to construct a standard curve for each assay plate. The standard curve was constructed by plotting the threshold cycle (C_T) versus the natural log of input RNA (ng). This curve was then used to calculate the relative abundance of each transcript in each sample. Transcript abundance values were then normalized to those of β-actin (20) to control differences in RNA and cDNA loading. We selected β-actin as the housekeeper gene after testing elongation factor (EF)1-α, hypoxanthine-guanine phosphoribosyltransferase (HPRT), 18S rRNA, and acidic ribosomal phosphoprotein (ARP). We had found that 18S, β-actin, and EF1-α were all consistent but selected β-actin because of a better real-time PCR efficiency and C_T closer to the transcripts of interest. Each sample was run in triplicate on a single plate. All data are presented as log base 2 (Log_2) of normalized fold change relative to the vehicle injected samples. We used the Log_2 to allow visualization of the fold change in the positive (upregulated) and negative (downregulated) where Log_2 of 0 equals no change in gene expression and 1 and −1 equal twofold up- and downregulation, respectively.

Real-time PCR analysis on 16 selected transcripts was used to validate the microarray measurements (Table 1 and Supplemental Table S1). All of the transcripts selected for confirmation were found to have a P value of <0.01 and a FDR of <0.2 (Tables 2–5). Eight were selected per tissue, four of which were found to be significantly different between the GH-injected and vehicle-injected fish by microarray analysis in the low-growth group and four in the high-growth group.

For RT-PCR primer design for transcripts identified from the microarray experiment, we used the actual rainbow trout sequence, or the most closely related sequence identified in the rainbow trout gene index if the match was >90% over the length of the clone sequence. However, if it was not possible to find a match, we used the Atlantic salmon clone sequence for primer design. GRASP clone ID, primer sequence, annealing temperature, and primer concentration are shown in Table 1. Data were collected from eight individuals (4 vehicle injected and 4 GH injected) from one of the low-growth rate families injected for the microarray experiment. A t-test was used to compare

Table 1. *Real-time PCR primer sequences and cycling parameters*

Feature ID	Annotation	Forward Primer	Concentration, nM	Reverse Primer	Concentration, nM	AT, °C	Size, bp
		Liver significance verification					
CB493973	Apolipoprotein B	AATGCTGATTGGCTGACGAG	400	CCATATACCACAAACCCCAGAG	200	64	110
CA061478	CA061478_Ssa	CAGAGAATGCCAGACTTTGATG	100	GGCATATTACATCATCAGCAGC	200	60	128
CB490453	DnaJ (Hsp40) homolog	GCGAGCACAGTGAGAATGAC	400	GATCTCCTTCTTCTGGGCAG	400	64	131
CB510638	Haptoglobin 2	CCGCTGAGTCACTGAAACAC	400	CTTGCTGGCTCCAGTACAGA	400	60	137
CA048996	PDIA 4	TCCACTTTGCCACTGTCATC	400	TCGTCGTTCAAGACCAACAC	400	60	148
CA046225	Metallothionein	TCCTGCAAGTGCTCAAACTG	400	GCACATTTACTGCAGTCGGA	400	60	83
CA059003	TC111082_Omy	GCAGACGTCTAGTTCATTCAGG	400	TCGGACACAAACTTGGACTG	400	56	140
CA039289	TC119763_Omy	CTAGCTGAACCAATCCCATGTC	400	TTAGCCAGCAAGCACCATTC	400	56	92
		Muscle significance verification					
CA055447	CA055447_Ssa	ATTCTAATGGACGTTCACCAGC	200	TCCAGACCAGTGTACATTTCCA	200	64	78
CB498293	Creatine kinase type B	CCAGAGATGTACTCCAACTTGC	400	ACAGCCCACTGTCATGATGA	400	56	117
CA504199	Gastrotropin	CATCATTGAGAAGGGTCGTG	200	TGTTTGTGGGATAGAGCTGG	200	62	92
CA057469	TC104650_Omy	TGGCTGCTCGTACTCGTATC	400	CTCCTCACATTGCAGTGCTC	400	64	132
CB488515	TC119728_Omy	AACCTGAACGAGCTCTTACCAG	400	GATTCGTTGATCTTGTCGCC	400	60	78
CB494048	Tubulin alpha-1	GAGTCCAGATGGGTAATGCC	400	AAGAGTCGTCTCCACCTCCA	400	60	108
CA051243	Ubiquitin-activating E1C	AAGCTGAGTTTGAAGAGCACAG	400	TGAAAGAGCTGGGTCTGTTAGA	400	62	85
		Selected gene expression					
CB509699	Adipose differentiation-related protein	AGCATCAAAAGCAATGTCCTGC	100	TGAATGTTGCTCAGTGTGTGCTC	100	58	81
CB506162	Dimethylanaline monooxygenase-like	TGTTGCTCCCACTGTGTGTCA	200	ATGGAGGCAGAGACAGGTTCAC	200	64	80
CA061211	NDRG3	GCTGCCACTAACCACACCTACAA	400	CAACCTCTTCGGCATAACTCTTTGG	400	66	78
CA061794	Transferrin receptor 1	CCGGTCTCAACATGTCCTA	400	CCTGAATGGTGAAGCCTAAC	400	54	63
CB500563	Twist	TTCCTACTATCATCACCGACA	400	ACAGTGTTGTATGACGCTTCT	400	54	83
	β-Actin	CAGCCCTCCTTCCTCGGTAT	400	AGCACCGTGTTGGCGTACA	400	60	110

AT, annealing temperature.

the expression level of the GH-treated group to the vehicle-injected group; P values of <0.05 were considered significant. We also verified the annotation of these transcripts as shown in Supplemental Table S1.

To place the results of our study in a broader context, we measured six growth-related transcripts (GHR1, GHR2, IGF-I, IGF-II, MSTN1A, MSTN1B) because they are important components of the growth regulatory axis; however, they were not found to be significant via microarray analysis. The primers for these transcripts were designed previously: GHRs (K. Nichols, unpublished observations), IGF-I and -II (19), and MSTNs (22). We also investigated the tissue specificity of the response to GH by comparing expression of 20 select transcripts across 8 tissues (liver, muscle, brain, gill, heart, intestine, kidney, stomach) in the GH-injected and vehicle-injected low-growth rate fish. Of these 20 genes, 15 were the transcripts used for verification above and the other 5 showed a strong trend in the microarray analysis and were selected because of their actions in lipid metabolism (adipose differentiation-related protein, dimethylanaline monooxygenase-like) or growth (n-myc downstream regulated gene 1, transferrin receptor, and twist-related protein 2). All of the tissues used for the real-time PCR analysis were collected from one low-growth rate family used for the microarray analysis ($n = 4$ for GH, $n = 4$ for vehicle). For all 20 transcripts, we had confirmed the annotation with both BLASTn and BLASTx searches of GenBank (shown in Supplemental Table S1). For transcripts that did not show a significant match to a previously identified protein or gene, the geneID was assigned as the sequence used for primer design (GenBank accession or gene index contig assignment, with species identification as Omy = *Oncorhynchus mykiss* and Ssa = *Salmo salar*).

RESULTS

Plasma IGF-I concentration. Plasma IGF-I was greater in the high-growth rate fish than in the low-growth rate fish ($P < 0.01$). Posilac injection significantly increased plasma IGF-I concentrations in both the high ($P < 0.01$)- and low ($P < 0.01$)-growth rate fish compared with vehicle-injected and uninjected control animals, and there were no differences between the vehicle-injected and uninjected control animals within growth rate (Fig. 1). Plasma IGF-I concentrations (means ± SE) from the high-growth rate fish were 209.3 ± 19.6, 94.0 ± 5.7, and 85.2 ± 7.9 ng/ml for GH-injected, vehicle-injected, and uninjected control animals, respectively. In the low-growth rate fish, the concentrations (means ± SE)

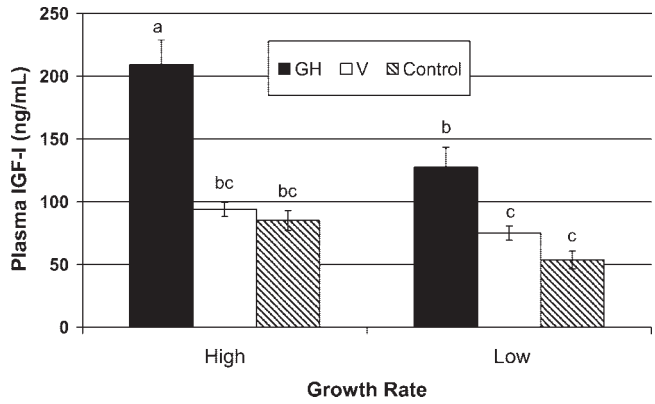

Fig. 1. Plasma concentration of insulin-like growth factor (IGF)-I in growth hormone (GH)-injected (GH), vehicle-injected (V), and untouched control (Control) groups. Samples are shown as means ± SE ($n = 8$ for GH and V, $n = 6$ for Control); columns with different superscript letters are significantly different ($P < 0.05$).

were 127.5 ± 15.9, 75.1 ± 5.7, and 53.7 ± 7.0 ng/ml for the same groups. The relative increases were approximately the same for both groups of fish: 246% for high-growth and 237% for low-growth fish.

Microarray expression analysis. The data from the microarray experiments are described below for each of the individual groups. The microarray data have been submitted to the Gene Expression Omnibus database (**http://www.ncbi.nlm.nih.gov/geo/**; accession nos. GSM189698, GSM189699, GSM189701, GSM189705, GSM189706, GSM189707, GSM189720. GSM189721, GSM189731, GSM189744–GSM189750). Data preprocessing identified all of the slides to be of "good" quality or better.

The hybridizations comparing the liver samples collected from the GH-injected and vehicle-injected low-growth rate fish revealed differences in 339 features with a P value <0.01 and with 42 having a FDR <0.2 (Table 2). Of these 42 features, 13 were upregulated and 29 were downregulated by exogenous GH. The hybridization with the RNA from the livers of the high-growth rate trout identified a total of 279 differentially expressed features with a P value <0.01. The lowest FDR for this group was 0.423. The high FDR was the result of a slide failure that resulted in an unbalanced number of slides for the dye swap analysis. In Table 3, we list the features identified with the lowest FDR. Of the 24 features, 13 were upregulated and 11 were downregulated. Transcripts associated with immune function and metabolism were among the most common of those altered by GH treatment for both low- and high-growth fish.

The hybridization comparing the muscle samples collected from the GH- and vehicle-injected low-growth fish identified 242 features with a P value <0.01, including 18 with a FDR <0.2 (Table 4). Of these features, 13 were identified as upregulated and 5 were downregulated by GH administration. The hybridization with RNA from the high-growth rate muscle samples identified a total of 422 features with a P value <0.01, including 96 with a FDR <0.2. These features included 71 upregulated transcripts and 25 downregulated transcripts (Table 5). Most common were transcripts associated with the cell cycle, immune function, and metabolism including protein metabolism. Surprisingly, there was no overlap in the complement of altered transcripts among the four groups.

Real-time PCR analysis confirmed the microarray results. Relative expression of the eight genes measured in the liver and the seven genes measured in the muscle of the low-growth fish were similar when measured by microarray or by real-time PCR (Figs. 2 and 3). We failed to develop a real-time PCR assay for one of the selected transcripts. For the liver and muscle all four transcripts that were significantly different in the low-growth fish by microarray were also significantly different by real-time PCR. Of the four liver transcripts that were significantly different in the high-growth group but not the low-growth group by microarray, only one, metallothionein, was significantly different in the low-growth group by

Table 2. *List of differentially expressed genes in liver of low-growth rate rainbow trout treated with GH*

ID	Name	Primary Function	Log_2 of FC	P Value	FDR
CB498736	Cysteine-rich protein 1	Cell cycle	−0.74	8.79E-06	0.016
CA044192	Programmed cell death protein 6	Cell cycle	−0.69	0.000392	0.157
CB490453	DnaJ homolog subfamily C member 7	Chaperone	0.68	2.07E-05	0.026
CB517240	Mitochondrial import receptor subunit TOM34	Chaperone	−0.59	0.000351	0.144
CB487733	Clusterin precursor	Immune	−0.53	0.000275	0.123
CA039257	Complement C1r subcomponent precursor	Immune	1.10	7.38E-05	0.066
CB510300	Complement C1r subcomponent precursor	Immune	1.36	1.44E-05	0.021
CB499529	Ferritin heavy subunit	Immune	−0.87	3.76E-05	0.043
CB510638	Haptoglobin	Immune	1.65	6.55E-09	0.0001
DN047719	Haptoglobin	Immune	1.29	4.59E-05	0.049
CA049856	Integrin beta-7 precursor	Immune	−0.80	7.93E-05	0.066
CB487219	Murinoglobulin 1 precursor	Immune	−0.78	4.91E-05	0.049
CK991302	*Oncorhynchus mykiss* calreticulin	Immune	0.45	0.00028	0.123
CB490652	*Oncorhynchus mykiss* iip1 (interferon inducible)	Immune	−0.90	0.000286	0.123
CB489380	Signal peptide peptidase-like 2A	Immune	−0.75	0.000128	0.094
CB509522	TSC22 domain family protein 3	Immune	−1.23	7.48E-06	0.016
CB494343	Adenosylhomocysteinase	Metabolism	0.72	9.66E-05	0.074
CB494476	Adenosylhomocysteinase	Metabolism	0.80	0.000141	0.094
CB493973	Apolipoprotein B	Metabolism	−1.33	8.77E-08	0.0007
CB497703	Apolipoprotein B	Metabolism	−1.17	1.94E-06	0.009
CB511166	Apolipoprotein B	Metabolism	−0.61	7.17E-05	0.066
CA042805	Cytochrome *P*-450 2D9	Metabolism	−0.48	0.000292	0.123
CB509870	Prostaglandin D synthase	Metabolism	−0.99	0.000455	0.174
CA048996	Protein disulfide-isomerase A4 precursor	Metabolism	0.86	2.26E-06	0.009
CA041920	*Danio rerio* ATPase, Na^+/K^+ transporting	Osmoregulation	0.51	0.000274	0.123
CB509453	60S ribosomal protein L3	Protein synthesis	−1.24	3.99E-06	0.011
CA043880	Rab5 GDP/GTP exchange factor	Signal transduction	−0.68	0.000158	0.1
CB488729	Cleavage and polyadenylation specificity factor	Splicing	−0.71	0.000428	0.168
CA046653	Similar to U2AF2 protein	Splicing	−0.79	0.000176	0.105
CB499390	Myosin heavy chain, smooth muscle isoform	Structural	−0.58	0.000198	0.114
CA041728	Myosin regulatory light chain 2	Structural	−0.94	0.000223	0.119
CB515401	cDNA DKFZp686E2449	Unknown	−0.62	0.000237	0.119
CB486684	Reverse transcriptase-like protein	Unknown	−0.93	8.46E-06	0.016

Unknowns: CA039186, CA039566, CA041061, CA048447, CA051670, CA054344, CA062992, CB502696, CB509472. GH, growth hormone; FC, fold change; FDR, false discovery rate.

Table 3. *List of differentially expressed genes in liver of high-growth rate rainbow trout treated with GH*

ID	Name	Primary Function	Log_2 of FC	*P* Value	FDR
CB516420	Inhibitor of growth protein 4	Cell cycle	0.42	6.09E-04	0.423
CB510488	Complement factor D precursor	Immune	−0.61	3.62E-04	0.423
CA057411	Ferritin heavy chain	Immune	−0.80	5.34E-04	0.423
CA045984	Cytochrome-*c* oxidase subunit 1	Metabolism	0.64	4.40E-04	0.423
CA038193	Gastrotropin (GT)	Metabolism	−0.65	6.00E-04	0.423
CB513900	Ribonuclease T2 precursor	Metabolism	0.44	4.08E-04	0.423
CA061478	Alpha-globin and beta-globin	O_2 transport	−0.60	2.33E-04	0.423
CB497309	Hemoglobin beta-2 chain	O_2 transport	−0.98	5.14E-04	0.423
CB493440	Proteasome subunit alpha type 4	Protein degradation	−0.55	3.79E-04	0.423
CB494678	60S ribosomal protein L35a	Protein synthesis	−0.67	6.33E-04	0.423
CA046225	Metallothionein-I	Stress response	−0.98	1.51E-04	0.423
CB497894	Similar to fibrinogen, beta chain	Structural	−0.79	2.39E-04	0.423
CA053936	Similar to mKIAA1931 protein	Unknown	0.57	3.42E-04	0.423

Unknowns: CA039289, CA052630, CA052760, CA059003, CA062231, CB500733, CB516108, CB516395, CB517777, CB517950, CK991306.

real-time PCR. In the muscle, two of three that were significant in the high-growth group but not the low-growth group by microarray, creatine kinase type B and TC104650, were significantly different in the low-growth group by real-time PCR; however, creatine kinase type B was altered in the opposite direction. In addition, the trend in direction of expression with the microarray and real-time PCR was the same metallothionein, creatine kinase type B, and TC104650 for the low-growth samples.

Multitissue modulation of gene expression. Figure 4 shows the effects of GH treatment on gene expression across the eight tissues collected from the low-growth fish. The complete real-time PCR data set is available in Supplemental Table S2. Two of the five transcripts that showed a nonsignificant trend in the microarray were significantly different by real-time PCR analysis.

Twist-related protein 2 was the only transcript that was not found to be significantly regulated in any tissue (Fig. 4). The transcripts TC104650, haptoglobin 2, tubulin-α1, TC111082, and adipose differentiation-related protein were found to be significantly different only when upregulated (Fig. 4). In contrast, CA055447, CA061478, and dimethylanaline monooxygenase-like transcripts were significantly different only when downregulated (Fig. 4). The remaining 11 transcripts showed differences in the direction of regulation among the tissues examined (Fig. 4). Four transcripts, apolipoprotein B, haptoglobin 2, gastrotropin, and twist-related protein 2, were not detectable in some tissues examined (Fig. 4).

Modulation of additional growth-related genes. After GH administration, the expression of both IGFs was significantly increased in the heart and liver and IGF-I was significantly reduced in the stomach (Fig. 5). Expression of GHR1 and -2 were both significantly increased in the liver, muscle, and stomach. Levels of GHR2 were also increased in the gill and brain and decreased in the kidney, whereas GHR1 was decreased in the brain (Fig. 6). MSTN1A and MSTN1B often showed opposite responses among tissues. MSTN1A was decreased and MSTN1B increased in the brain, whereas the opposite was found in muscle. Only MSTN1A was decreased in the liver, and only MSTN1B was decreased in the kidney (Fig. 7).

DISCUSSION

Previous experiments have shown that exogenous GH treatment and the production of GH transgenic fish result in a significant increase in overall growth rate in rainbow trout and other salmonids (21, 39, 52). In addition, studies have examined the effects of GH augmentation on aspects of physiology and gene expression in salmonids (3, 4, 26, 44, 57, 66). In the present study, we characterized the short-term effects of exogenous GH on gene expression in the liver and the muscle of rainbow trout (*Oncorhynchus mykiss*), using a combination of microarray analysis and real-time PCR verification. This approach allows for a broader view of the impact of GH treatment than seen in previous studies. The GRASP 16K array is the

Table 4. *List of differentially expressed genes in muscle of low-growth rate rainbow trout treated with GH*

ID	Name	Primary Function	Log_2 of FC	*P* Value	FDR
CB498021	Heat shock protein HSP 90-alpha (HSP 86)	Chaperone	0.90	4.33E-05	0.099
CA043744	Telomerase-binding protein p23	Chaperone	0.71	1.32E-04	0.193
CB497820	ADP,ATP carrier protein 2	Metabolism	0.53	2.23E-04	0.199
CB510571	Apolipoprotein A-IV precursor	Metabolism	0.57	1.76E-05	0.081
CB497434	Apolipoprotein CII	Metabolism	0.86	1.30E-04	0.193
CB488180	ATP synthase D chain, mitochondrial	Metabolism	0.59	1.61E-04	0.198
CB504199	Gastrotropin (GT)	Metabolism	−0.62	1.77E-04	0.199
CB488515	Thioredoxin-related transmembrane protein 2	Metabolism	0.88	3.27E-07	0.005
CB488716	High choriolytic hatching proteinase	Protein degradation	−0.50	2.10E-04	0.199
CB510845	Beta crystallin B2	Structural	0.91	2.16E-04	0.199
CB494048	Tubulin alpha-1 chain	Structural	0.70	2.03E-05	0.081

Unknowns: CA048050, CA055447, CA057495, CA058184, CA058748, CB485975, CB510800.

Table 5. *List of differentially expressed genes in muscle of high-growth rate rainbow trout treated with GH*

ID	Name	Function	Log_2 of FC	*P* Value	FDR
CA058262	Similar to apoptosis inhibitor 5	Antiapoptosis	0.61	7.11E-04	0.164
CK991293	Cyclin G1 (CCNG1)	Cell cycle	−0.44	7.65E-04	0.164
CA063201	Dual-specificity protein kinase TTK	Cell cycle	0.60	4.82E-04	0.143
CA051116	Flap endonuclease-1	Cell cycle	0.49	3.69E-04	0.134
CB496359	Similar to CDC26 subunit	Cell cycle	0.58	7.60E-04	0.164
CA060993	S-phase kinase-associated protein 1A	Cell cycle	0.62	1.57E-04	0.112
CA051243	Ubiquitin-activating enzyme E1c	Cell cycle	0.65	1.23E-05	0.045
CA053164	Sacsin	Chaperone	−0.45	1.04E-04	0.101
CK991042	Somatolactin	Hormone	0.55	6.54E-04	0.161
CA043104	Acidic mammalian chitinase precursor	Immune	−0.65	3.52E-04	0.131
CA057098	Barrier-to-autointegration factor	Immune	0.80	1.38E-05	0.045
CB516917	CCAAT/enhancer binding protein epsilon	Immune	0.46	6.61E-04	0.161
CB510024	D-dopachrome tautomerase	Immune	0.69	1.07E-04	0.101
CK990254	Human G protein-coupled receptor (GPR2)	Immune	−0.43	3.75E-04	0.134
CB516783	Transforming protein RhoA	Immune	0.56	5.68E-04	0.152
CB514566	Tripartite motif protein 39	Immune	0.67	4.72E-04	0.143
CB494098	Alpha-glucosidase I	Metabolism	0.49	8.38E-04	0.173
BU965693	ATP synthase coupling factor 6	Metabolism	0.65	3.96E-05	0.079
CB498293	Creatine kinase, B chain	Metabolism	−0.54	5.47E-05	0.088
CB494403	Creatine kinase, M chain	Metabolism	−0.49	1.02E-03	0.185
CA064095	Endonuclease G, mitochondrial precursor	Metabolism	1.19	4.79E-04	0.143
CA058986	Formimidoyltransferase-cyclodeaminase	Metabolism	0.56	7.50E-04	0.164
CA041773	Mitochondrial Tim22	Metabolism	0.42	6.35E-04	0.161
CB512542	Succinyl-CoA ligase alpha-chain	Metabolism	0.84	4.88E-04	0.143
CA064277	Hemoglobin alpha chain.	O_2 transport	0.62	2.15E-04	0.115
CA056168	Vasoactive cardiac hormone	Osmoregulation	0.70	1.69E-05	0.045
CB516784	26S protease regulatory subunit 6B	Protein degradation	0.57	9.04E-04	0.175
CA059453	Ankyrin repeat and SOCS box protein 9	Protein degradation	0.60	4.99E-04	0.143
CK990334	F-box only protein 9	Protein degradation	0.53	4.78E-04	0.143
CB499136	F-box protein 7	Protein degradation	0.72	2.26E-04	0.115
CA053799	Ubiquitin specific protease 48	Protein degradation	0.67	1.88E-04	0.115
CB491207	40S ribosomal protein S23.	Protein synthesis	0.85	1.07E-03	0.189
CB496752	40S ribosomal protein S27	Protein synthesis	0.71	3.17E-04	0.129
CB515185	40S ribosomal protein SA	Protein synthesis	0.41	7.00E-04	0.164
CA770456	60S ribosomal protein L10	Protein synthesis	0.77	3.29E-04	0.129
CK990556	60S ribosomal protein L13	Protein synthesis	−0.59	3.37E-05	0.077
CA049278	60S ribosomal protein L22	Protein synthesis	0.43	9.76E-04	0.181
CB498552	60S ribosomal protein L5.	Protein synthesis	0.49	6.51E-04	0.161
CB508429	Ribonuclease P protein subunit p21	Protein synthesis	−0.64	7.53E-04	0.164
CB517429	ATP-dependent RNA helicase WM6.	Splicing	0.51	1.17E-03	0.195
CK991026	Heterogeneous nuclear ribonucleoprotein M	Splicing	0.56	1.03E-03	0.185
CA042976	Ribosome associated membrane protein 4	Stress response	0.65	2.29E-04	0.115
CA058991	Claudin 12	Structural	0.82	2.16E-04	0.115
CA063101	Gamma tubulin ring complex protein	Structural	0.69	7.20E-04	0.164
BU965651	Myosin light polypeptide 3	Structural	0.59	9.42E-04	0.18
CK991313	*Oncorhynchus keta* mRNA for actin	Structural	−0.69	2.53E-04	0.117
CB497986	Troponin T, fast skeletal muscle isoforms.	Structural	−0.54	2.98E-04	0.129
CB498116	Troponin T, fast skeletal muscle isoforms.	Structural	−0.57	7.09E-04	0.164
CK991338	Synaptosome-associated protein 25a	Synapse	0.77	1.11E-03	0.19
CA052188	Similar to liver nuclear protein	Transcription	−0.52	9.06E-04	0.175
CA043389	Transcription factor BTF3	Transcription	0.60	5.65E-04	0.152
CB496929	Bleomycin hydrolase	Unknown	0.60	7.67E-05	0.088
CA056781	CGI-90 protein	Unknown	0.54	6.19E-05	0.088
CB510043	Coiled-coil-helix-coiled-coil-helix domain 2	Unknown	0.66	9.78E-04	0.181
CA056250	G patch domain containing protein 3.	Unknown	−0.53	7.48E-05	0.088
CB516141	P17F11 protein	Unknown	0.77	3.28E-04	0.129
CA055427	Transposase	Unknown	−0.73	1.17E-04	0.104

Unknowns: CA041408, CA042169, CA043781, CA051041, CA051184, CA052167, CA052578, CA053450, CA053452, CA054034, CA054293, CA054425, CA055185, CA055638, CA056260, CA056919, CA057469, CA057894, CA057909, CA058267, CA060269, CA062038, CA062412, CA062670, CA063549, CB500559, CB501853, CB507270, CB509073, CB510423, CB511056, CB514817, CB518118, CK990506, CK990527, CK990570, CK990737, CK991267, CK991270.

broadest platform available for salmonid gene expression analysis, but the lack of a genomic sequence in salmonids means that it is not comprehensive. For this reason we supplemented our array analyses by measuring certain key genes by real-time PCR. Furthermore, real-time PCR was used to verify significant findings of the microarray and also to reevaluate interesting trends in key genes that were not significant with the microarray investigation.

We chose to sample 3 days after a sustained GH-releasing implant (Posilac, Monsanto) to determine the modulation of

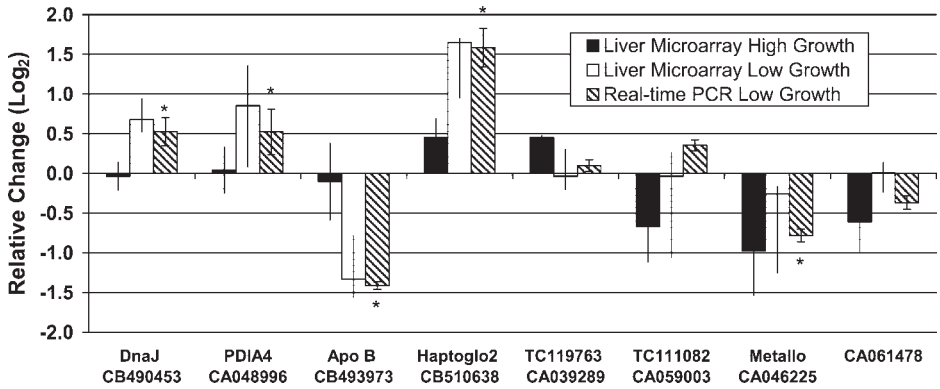

Fig. 2. Real-time PCR verification of 8 transcripts identified as significantly different after GH treatment in the liver via microarray analysis. All means are shown as the Log_2 of relative change compared with the vehicle-injected samples, with the microarray error bars (uncapped) showing the range across the microarray slides and the real-time PCR error bars showing SE. Transcripts with GenBank accession numbers: DnaJ (Hsp40) homolog (DnaJ), protein disulfide isomerase associated 4 (PDIA4), apolipoprotein B (ApoB), haptoglobin 2 (Haptoglo2), TC119763_Omy, TC11082_Omy, metallothionein (Metallo), and CA061478_Ssa. *Real-time PCR values significantly different between GH-injected and vehicle-injected control samples ($P < 0.05$).

gene expression in the metabolic center (liver) and the marketable product (muscle) of the rainbow trout. The dosage and the treatment time were selected based on reported plasma IGF-I response to the same GH preparation (4). A primary action of GH is the stimulation of IGF-I production by the liver (5, 41). We used elevated serum IGF-I to confirm the stimulation of the GH/IGF-I system in Posilac-treated rainbow trout (Fig. 1). Although the duration of our study was too short to attempt to detect changes in growth, we observed a increase in plasma IGF-I similar to that reported in a previous experiment in which growth was significantly increased by Posilac injections over a longer time frame (4).

We report 66 and 114 genes that are differentially expressed in the liver and muscle, respectively, after short-term GH treatment. The largest portion of these transcripts includes those listed as having undefined functions in cellular processes. This was not unexpected, because neither Atlantic salmon nor rainbow trout genomes have been fully sequenced and, therefore, a large number of spots on the chip are uncharacterized EST sequences. In addition, previous microarray experiments in the rainbow trout have identified as altered a large number of uncharacterized transcripts (51, 46).

For real-time PCR verification of our microarray findings, we selected a total of 16 genes (4 from each treatment group) observed to be significantly differentially regulated after GH administration (Table 1, Figs. 2 and 3). We designed primers and successfully assayed 15 of these transcripts and found them to have a level and a direction of expression similar to the microarray data. All differences found to be significant by microarray were confirmed by real-time PCR, that is, comparing the samples within the low-growth rate group. However, three of seven transcripts that were not identified as significantly altered in the low-growth rate samples when examined by microarray were found to be significantly different when measured by real-time PCR (Figs. 2 and 3). The directions of modulation of two of the three genes, metallothionein and TC104650, were similar for microarray and real-time PCR. Similarly, when five additional transcripts (Fig. 4) found to have trends approaching significance via microarray measurements were examined by real-time PCR, two of these trends proved significant (Fig. 4). It is not unexpected that real-time PCR is able to detect changes that are not detected by microarray, because real-time PCR is a more sensitive method for measuring transcripts and there is greater statistical power

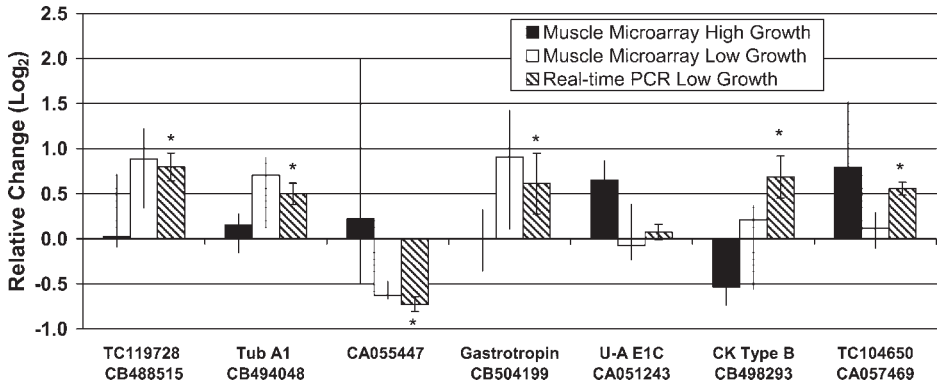

Fig. 3. Real-time PCR verification of 8 transcripts identified as significantly different after GH treatment in the muscle via microarray analysis. All means are shown as the Log_2 of relative change compared with the vehicle-injected samples, with the microarray error bars (uncapped) showing the range across the microarray slides and the real-time PCR error bars showing SE. Transcripts with GenBank accession numbers: TC119728_Omy, tubulin alpha 1 (Tub A1), CA055447_Ssa, gastrotropin, ubiquitin-activating enzyme E1C (U-A E1C), creatine kinase type B (CK Type B), and TC104650_Omy. *Real-time PCR values significantly different between GH-injected and vehicle-injected control samples ($P < 0.05$).

GB No.	Gene ID	Brain	Gill	Heart	Intestines	Kidney	Liver	Muscle	Stomach
Liver Significance Verification									
CB493973	Apolipoprotein B	ND	ND		ND	ND		ND	ND
CA061478	CA061478_Ssa								
CB490453	DnaJ homolog								
CB510638	Haptoglobin 2		ND		ND	ND			
CA046225	Metallothionein								
CA048996	PDIA4								
CA059003	TC111082_Omy								
CA039289	TC119763_Omy								
Muscle Significance Verification									
CA055447	CA055447_Ssa								
CB498293	Creatine Kinase Type B								
CB504199	Gastrotropin		ND	ND					ND
CA057469	TC104650_Omy								
CB488515	TC119728_Omy								
CB494048	Tubulin Alpha 1								
CA051243	Ubiquitin-Activating Enzyme E1C								
Selected Gene Expression									
CB509699	Adipose Differentiation-Related protein								
CB506162	Dimethylanaline monooxygenase-like								
CA061211	N-myc downstream regulated gene 1								
CA061794	Transferrin Receptor								
CB500563	Twist-Related Protein 2	ND		ND	ND	ND	ND		ND

Fig. 4. Heat diagram of differential expression of selected transcripts across 8 tissues after GH administration as measured by real-time PCR. Black blocks, significant upregulation; gray blocks, significant downregulation; white blocks, no significant difference; ND, no transcript detectable.

when comparing fewer transcripts (45, 65). Creatine kinase type B (in the muscle) and TC111082 (in the liver) transcripts were determined to have expression patterns via real-time PCR different from those observed on the microarray (Figs. 2 and 3). We suspect this is the result of cross-hybridization with closely related transcripts, as previously suggested by Kothapalli et al. (33). However, these results show consistent results comparing the microarray and the real-time PCR within the growth rate.

In this study, we looked at the modulation of gene expression in response to GH treatment in rainbow trout selected for high growth rate or low growth rate. Surprisingly, GH treatment did not produce the same microarray features in the muscle or liver from both the high- and low-growth rate families. There are several possible factors contributing to this difference in response. These include activation of different genes between the growth phenotypes or differences in the magnitude of response resulting in changes in the ability to detect differences between the phenotypes. There is support for both notions in our study. For example, based on the microarray data, creatine kinase type B was significantly downregulated in muscle of the high-growth fish and unaltered in the low-growth fish. However, real-time PCR analysis revealed a significant upregulation of creatine kinase type B in the muscle of the low-growth phenotype. This indicates opposite responses to GH by the two phenotypes. On the other hand, microarray analysis showed that the transcript TC104650 increased in the muscle of high-growth fish and was unchanged in the low-growth fish, but real-time PCR analysis supports a significant increase in the low-growth muscle as well. Similarly, liver metallothionein was significantly downregulated in response to GH in the high-growth fish and unaltered in the low-growth fish based on microarray analysis; however, real-time PCR analysis showed a downregulation in the liver of the low-growth phenotype. Obviously, these differences were not detected by the less sensitive method of microarray analysis in these tissues from the low-growth phenotype. In addition, larger differences in expression in the high-growth fish that were significant based on microarray analysis showed similar trends with real-time PCR in the low-growth fish, such as with muscle ubiquitin-activating enzyme E1C and liver TC11973 and CA061478. We only analyzed the samples for relative expression and did not compare controls or GH effects between the two phenotypes to further reveal the basis for differences in the sets of features altered by GH treatment between the low-growth and high-growth phenotypes.

We measured the gene expression levels of a total of 26 genes across 8 tissues by real-time PCR for comparison to the responses of the liver and muscle to GH treatment. There was a high degree of variability in transcript response among tissues. However, in general, those genes identified as being differentially regulated in the muscle were found to have a similar expression pattern in the other tissues (Figs. 4–7). Excluding the liver, six genes that were identified as upregu-

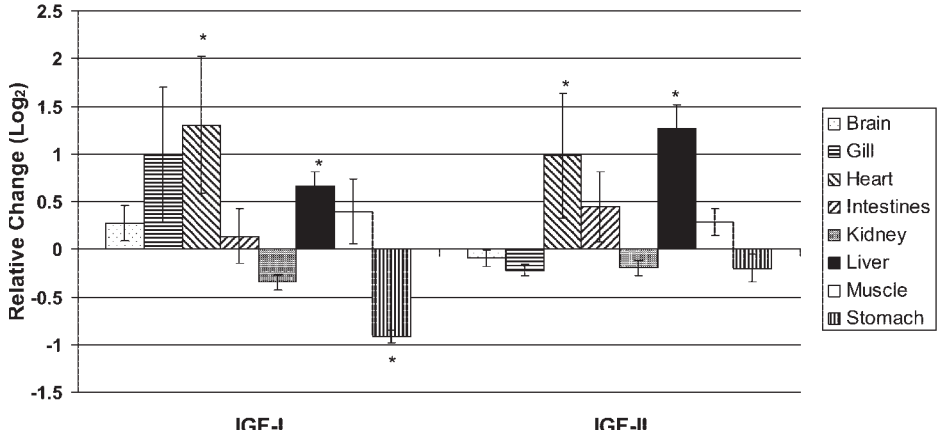

Fig. 5. Changes in IGF-I and IGF-II transcript following GH administration in brain, gill, heart, intestines, kidney, liver, muscle, and stomach as measured by real-time PCR. All means are shown as the $Log_2 \pm$ SE of fold change (1 = 2-fold change) compared with vehicle-injected samples. *Real-time PCR values significantly different between GH-injected and vehicle-injected control samples ($P < 0.05$).

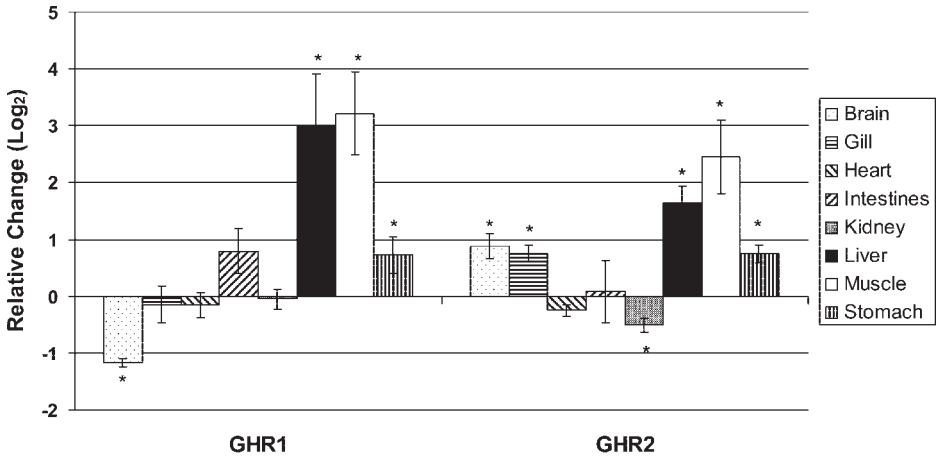

Fig. 6. Changes in growth hormone receptor (GHR)1 and GHR2 transcripts following GH administration in brain, gill, heart, intestines, kidney, liver, muscle, and stomach as measured by real-time PCR. All means are shown as $Log_2 \pm$ SE of relative change compared with vehicle-injected samples. *Real-time PCR values significantly different between GH-injected and vehicle-injected control samples ($P < 0.05$).

lated in muscle via real-time PCR were also only identified as significantly upregulated in other tissues, and one of the three downregulated genes in muscle was also downregulated in all other tissues in which there was a change (Fig. 4). This may suggest a common effect of either GH or IGF-I in the extrahepatic sites. In a GH transgenic tilapia, Eppler et al. (16) reported common extrahepatic modulation of gene expression.

Two recent papers have used microarrays to compare the gene expression in the liver of GH transgenic coho salmon (GRASP microarray) (49) and GH transgenic amago salmon (cDNA subtraction and microarray) (41) compared with their nontransgenic siblings. In both studies, a number of immune-related genes were identified as being differentially regulated. In this study, we found that 21 immune-related genes were differentially regulated across all treatment groups, with 14 of these genes being identified in the liver. Similar to the transgenic fish, we found modulation of the complement factor genes, CCAAT/enhancer-binding protein, and barrier-to-autointegration factor genes via microarray. These are in agreement with previous studies that observed increased immune stimulation following GH administration (4) or seawater acclimation, in which endogenous GH is typically elevated (56, 66). However, in the present study, we have also identified immune-related genes that that were not differentially expressed in the GH transgenic fish, indicating a difference in the response between the short-term and long-term effects of GH on the immune system.

In mammals exogenous GH administration results in decreased fat deposition and increased lipolysis (9). Similarly, Norbeck et al. (43) demonstrated significant mobilization of lipids in rainbow trout following a 6-wk fast, which correlated with an increased expression of the GHR in the adipose tissue but not in other tissues. They concluded that the increased signaling by GH in the adipose tissue acts to increase the mobilization of lipids. However, there is little evidence showing the effect of GH on lipid metabolic gene expression in the fed rainbow trout. In the present study, we have identified a number of lipid metabolism-related genes differentially expressed after GH treatment.

We observed a decrease in apolipoprotein B (ApoB) mRNA in the liver and increases in ApoCII and ApoAIV in the muscle of the low-growth phenotype. The different levels of the Apo transcripts are likely a reflection of their unique functions. The reduction of the ApoB expression in the liver could be an indication of reduced export of triglycerides from this tissue (1). Additionally, the increased expression of the ApoCII, known to activate the lipoprotein lipase (55) in the muscle, may be an indication of the uptake of lipids by the muscle immediately after the GH treatment. We have identified modulation of Apo genes only in the low-growth fish, which may be an indication of differences in energy utilization between the high- and low-growth phenotypes used in the present study and may provide some indication for the basis of the difference in growth rates of these families. In addition to the apolipopro-

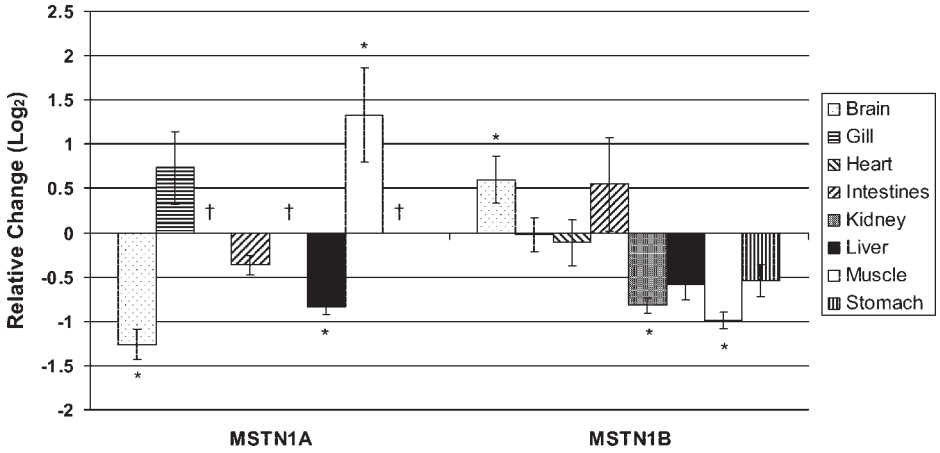

Fig. 7. Changes in myostatin (MSTN)1A and MSTN1B transcripts following GH administration in brain, gill, heart, intestines, kidney, liver, muscle, and stomach measured by real-time PCR. All means are shown as the $Log_2 \pm$ SE of relative change compared with vehicle-injected samples. *Real-time PCR values significantly different between GH-injected and vehicle-injected control samples ($P < 0.05$); †not detectable.

teins, upregulation of gastrotropin in the low-growth muscle and the high-growth liver further suggests modified lipid metabolism following GH treatment.

If we look at the tissue distribution of ApoB and gastrotropin in the low-growth rate fish after GH administration, we find further evidence of a complex relationship between lipid utilization and GH administration. The expression of ApoB was limited to the liver and the heart, and the expression was found to be significant in both but in opposite directions (down in the liver and up in the heart). We also determined the tissue distribution of a gastrotropin transcript. The combined upregulation of gastrotropin in the muscle and kidney and downregulation in the liver may suggest an increase in lipid uptake by the muscle and kidney and reduced uptake by the liver, consistent with the Apo data discussed above. These data suggest that, independent of nutritional status, GH treatment results in a significant alteration in lipid metabolism and utilization in rainbow trout.

Our measurements of growth-related gene expression confirm results of several previous studies and provide several new significant findings including GH-induced altered regulation of GHR1 and -2 receptor in muscle, liver, stomach, and brain in fish. As previously reported (5), we found a significant upregulation of IGF-I mRNA in the liver after GH administration. This is supported by increased plasma IGF-I. We also report a significant upregulation of IGF-II mRNA in the liver after GH administration. Upregulation of IGF-II expression by GH was shown previously in rainbow trout (53). Interestingly, Shamblott et al. (54) found that the increased expression of the IGF-II gene following GH administration was stimulated through the C/EBP sites in the gene promoter region. We also report that the C/EBP transcript (on the microarray) was upregulated after GH administration, suggesting a growth-promoting role for this transcript. In the present study, only the heart was found to have significant increases in IGF-I and -II outside the liver, and stomach IGF-I expression was significantly lower in the GH-treated individuals. We did not observe any changes in IGF expression in brain, muscle, kidney, and intestine, as found in carp (64). This discrepancy may be explained by the form of GH used, native versus a slow-release (heterologous) form; the time course (6 h vs. 3 days of treatment); or species differences.

The effects of GH on the target tissue were mediated through the GHRs. As mentioned above, we found significant upregulation of both GHR (1 and 2) transcripts in the liver, muscle, and stomach after GH administration. In mammals, previous studies reported that exogenous GH increased expression of both GHRs and levels of growth hormone binding protein (GHBP) (7, 12). The mammalian GHBP is made by either proteolytic cleavage of the GHR extracellular domain (human and rabbit) or as a splice variant of the GHR gene (mouse and rat) (18). Neither the transcript identity nor the mechanisms of GHBP formation have been reported in the rainbow trout. However, during saltwater acclimation in the rainbow trout, which is characterized by a significant increase in GH secretion (56), serum GHBP was found to increase significantly (62). Here we provide evidence for regulation of GHR or GHBP in fish similar to that indicated in mammalian species.

Myostatin is a member of the TGF-β superfamily, determined in mammals to be a potent regulator of skeletal muscle growth (40). Recent evidence in humans suggests a negative relationship between the GH responsiveness of a tissue and expression of myostatin gene in the muscle (37, 38). In the rainbow trout, three myostatin transcripts have been characterized (MSTN1A, -1B, -2A) and found to be expressed in a variety of tissues (22, 23, 48). Biga et al. (3) recently found that MSTN1A (reported as MSTN1) expression was higher and MSTN1B (reported as MSTN2) expression was lower in the muscle of rainbow trout after GH treatment. In GH transgenic coho salmon, Roberts et al. (50) found a significant reduction in MSTN1B (reported as MSTN2) in the white muscle but no change in the expression level of MSTN1A (reported as MSTN1) in the same tissue. This may indicate that the effects of GH on MSTN1A expression are transient in nature whereas the changes in MSTN1B are maintained with continued GH elevation.

In addition to the changes in the muscle, we observed that GH altered expression of MSTN1A in the brain and liver and of MSTN1B in the brain and kidney. Of particular interest was the downregulation of MSTN1A and upregulation of MSTN1B in the brain, whereas in the muscle we found upregulation of MSTN1A and downregulation of MSTN1B. Garikipati et al. (23) found that MSTN1A expression was greater than MSTN1B in rainbow trout white muscle and MSTN1A expression was lower in the brain. Therefore, these data indicate an enhancement of the differential expression of these MSTN genes in the muscle and brain with GH treatment, and may indicate a significantly different role for the MSTN protein in the regulation of growth and development in the rainbow trout as previously reported in mammalian species (10, 35).

In summary, we have shown that short-term (3 day) GH augmentation alters the expression of genes involved in metabolism, immune function, and growth regulation in rainbow trout. These findings are similar to those reported in GH transgenic salmon (49). However, some of the responses of genes in our short-term study were opposite in direction to those observed in the transgenic salmon, perhaps signifying differences between acute and chronic response to GH or species differences. Among the growth axis-related genes, we are the first to report an upregulation of rainbow trout GHR1 and -2 genes in the liver, muscle, and stomach following GH administration. The modulation of the MSTN genes following GH administration provides an indication of the modulation of genes regulating cell proliferation following GH administration. This study represents the first substantial characterization of rainbow trout liver and muscle transcript modulation after a short-term GH administration and will provide a wealth of information on the genes regulated by GH in the muscle and liver.

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REFERENCES

1. **Avramoglu RK, Adeli K.** Hepatic regulation of apolipoprotein B. *Rev Endocr Metab Disord* 5: 293–301, 2004.

2. **Benjamini Y, Hochberg Y.** Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc B* 57: 289–300, 1995.
3. **Biga PR, Cain KD, Hardy RW, Schelling GT, Overturf K, Roberts SB, Goetz FW, Ott TL.** Growth hormone differentially regulates muscle myostatin1 and -2 and increases circulating cortisol in rainbow trout (*Oncorhynchus mykiss*). *Gen Comp Endocrinol* 138: 32–41, 2004.
4. **Biga PR, Peterson BC, Schelling GT, Hardy RW, Cain KD, Overturf K, Ott TL.** Bovine growth hormone treatment increased IGF-I in circulation and induced the production of a specific immune response in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* 246: 437, 2005.
5. **Biga PR, Schelling GT, Hardy RW, Cain KD, Overturf K, Ott TL.** The effects of recombinant bovine somatotropin (rbST) on tissue IGF-I, IGF-I receptor, and GH mRNA levels in rainbow trout, *Oncorhynchus mykiss*. *Gen Comp Endocrinol* 135: 324–333, 2004.
6. **Bjornsson BT, Johansson V, Benedet S, Einarsdottir IE, Hildahl J, Agustsson T, Jönsson E.** Growth hormone endocrinology of salmonids: regulatory mechanisms and mode of action. *Fish Physiol Biochem* 27: 227–242, 2002.
7. **Brameld JM, Atkinson JL, Saunders JC, Pell JM, Buttery PJ, Gilmour RS.** Effects of growth hormone administration and dietary protein intake on insulin-like growth factor I and growth hormone receptor mRNA expression in porcine liver, skeletal muscle, and adipose tissue. *J Anim Sci* 74: 1832–1841, 1996.
8. **Butler AA, LeRoith D.** Tissue-specific versus generalized gene targeting of the igf1 and igf1r genes and their roles in insulin-like growth factor physiology. *Endocrinology* 142: 1685–1688, 2001.
9. **Butler AA, Roith DL.** Control of growth by the somatropic axis: growth hormone and the insulin-like growth factors have related and independent roles. *Annu Rev Physiol* 63: 141–164, 2001.
10. **Carnac G, Ricaud S, Vernus B, Bonnieu A.** Myostatin: biology and clinical relevance. *Mini Rev Med Chem* 6: 765–770, 2006.
11. **Chomczynski P, Sacchi N.** Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem* 162: 156–159, 1987.
12. **Combes S, Louveau I, Bonneau M.** Effect of GH administration on GH and IGF-I receptors in porcine skeletal muscle and liver in relation to plasma GH-binding protein. *J Endocrinol* 155: 19–26, 1997.
13. **Coulibaly I, Gahr SA, Palti Y, Yao J, Rexroad CE 3rd.** Genomic structure and expression of uncoupling protein 2 genes in rainbow trout (*Oncorhynchus mykiss*). *BMC Genomics* 7: 203, 2006.
14. **Devlin RH, Yesaki TY, Donaldson EM, Du SJ, Hew CL.** Production of germline transgenic Pacific salmonids with dramatically increased growth performance. *Can J Fish Aquat Sci* 52: 1376–1384, 1995.
15. **Du SJ, Gong ZY, Fletcher GL, Shears MA, King MJ, Idler DR, Hew CL.** Growth enhancement in transgenic Atlantic salmon by the use of an "all fish" chimeric growth hormone gene construct. *Biotechnology* 10: 176–181, 1992.
16. **Eppler E, Caelers A, Shved N, Hwang G, Rahman AM, Maclean N, Zapf J, Reinecke M.** Insulin-like growth factor I (IGF-I) in a growth-enhanced transgenic (GH-overexpressing) bony fish, the tilapia (*Oreochromis niloticus*): indication for a higher impact of autocrine/paracrine than of endocrine IGF-I. *Transgenic Res* 16: 479–489, 2007.
17. **Fauconneau B, Andre S, Chmaitilly J, Bail PY, Krieg F, Kaushik SJ.** Control of skeletal muscle fibres and adipose cells size in the flesh of rainbow trout. *J Fish Biol* 50: 296–314, 1997.
18. **Fisker S.** Physiology and pathophysiology of growth hormone-binding protein: methodological and clinical aspects. *Growth Horm IGF Res* 16: 1–28, 2006.
19. **Gabillard JC, Weil C, Rescan PY, Navarro I, Gutierrez J, Le Bail PY.** Effects of environmental temperature on IGF1, IGF2, and IGF type I receptor expression in rainbow trout (*Oncorhynchus mykiss*). *Gen Comp Endocrinol* 133: 233–242, 2003.
20. **Gahr SA, Rodriguez MF, Rexroad CE 3rd.** Identification and expression profile of the ID gene family in the rainbow trout (*Oncorhynchus mykiss*). *Biochim Biophys Acta* 1729: 64–73, 2005.
21. **Garber MJ, DeYonge KG, Byatt JC, Lellis WA, Honeyfield DC, Bull RC, Schelling GT, Roeder RA.** Dose-response effects of recombinant bovine somatotropin (Posilac) on growth performance and body composition of two-year-old rainbow trout (*Oncorhynchus mykiss*). *J Anim Sci* 73: 3216–3222, 1995.
22. **Garikipati DK, Gahr SA, Roalson EH, Rodgers BD.** Characterization of rainbow trout myostatin-2 genes (rtMSTN-2a and -2b): genomic organization, differential expression, and pseudogenization. *Endocrinology* 148: 2106–2115, 2007.
23. **Garikipati DK, Gahr SA, Rodgers BD.** Identification, characterization, and quantitative expression analysis of rainbow trout myostatin-1a and myostatin-1b genes. *J Endocrinol* 190: 879–888, 2006.
24. **Gentleman R, Carey VJ, Doudoit S, Irizarry RA, Huber W.** *Bioinformatics and Computational Biology Solutions Using R and Bioconductor*. New York: Springer, 2005.
25. **Gentleman RC, Carey VJ, Bates DM, Bolstad B, Dettling M, Dudoit S, Ellis B, Gautier L, Ge Y, Gentry J, Hornik K, Hothorn T, Huber W, Iacus S, Irizarry R, Leisch F, Li C, Maechler M, Rossini AJ, Sawitzki G, Smith C, Smyth G, Tierney L, Yang JY, Zhang J.** Bioconductor: open software development for computational biology and bioinformatics. *Genome Biol* 5: R80, 2004.
26. **Gomez JM, Weil C, Ollitrault M, Le Bail PY, Breton B, Le Gac F.** Growth hormone (GH) and gonadotropin subunit gene expression and pituitary and plasma changes during spermatogenesis and oogenesis in rainbow trout (*Oncorhynchus mykiss*). *Gen Comp Endocrinol* 113: 413–428, 1999.
27. **Green H, Morikawa M, Nixon T.** A dual effector theory of growth-hormone action. *Differentiation* 29: 195–198, 1985.
28. **Hernandez Llorente MD, Dato Gomez MJ, de Costa Ruiz J, Mendiola Lopez P, Zamora Navarro S.** Effect of recombinant human GH and GHRH on plasma metabolite levels in rainbow trout (*Oncorhynchus mykiss*). *J Physiol Biochem* 60: 211–218, 2004.
29. **Higgs DA, Donaldson EM, Dye HM, McBride JR.** A preliminary investigation of the effect of bovine growth hormone on growth and muscle composition of coho salmon (*Oncorhynchus kisutch*). *Gen Comp Endocrinol* 27: 240–253, 1975.
30. **Isaksson OG, Lindahl A, Nilsson A, Isgaard J.** Mechanism of the stimulatory effect of growth hormone on longitudinal bone growth. *Endocr Rev* 8: 426–438, 1987.
31. **Johansen KA, Sealey WM, Overturf K.** The effects of chronic immune stimulation on muscle growth in rainbow trout. *Comp Biochem Physiol B Biochem Mol Biol* 144: 520–531, 2006.
32. **Komourdjian MP, Burton MP, Idler DR.** Growth of rainbow trout, *Salmo gairdneri*, after hypophysectomy and somatotropin therapy. *Gen Comp Endocrinol* 34: 158–162, 1978.
33. **Kothapalli R, Yoder SJ, Mane S, Loughran TP Jr.** Microarray results: how accurate are they? *BMC Bioinformatics* 3: 22, 2002.
34. **Leatherland JF, Nuti RN.** Effects of bovine growth hormone on plasma FFA concentrations and liver, muscle and carcass lipid content in rainbow trout, *Salmo gairdneri* Richardson. *J Fish Biol* 19: 487–498, 1981.
35. **Lee SJ.** Regulation of muscle mass by myostatin. *Annu Rev Cell Dev Biol* 20: 61–86, 2004.
36. **Lee SJ, McPherron AC.** Myostatin and the control of skeletal muscle mass. *Curr Opin Genet Dev* 9: 604–607, 1999.
37. **Liu W, Thomas SG, Asa SL, Gonzalez-Cadavid N, Bhasin S, Ezzat S.** Myostatin is a skeletal muscle target of growth hormone anabolic action. *J Clin Endocrinol Metab* 88: 5490–5496, 2003.
38. **Marcell TJ, Harman SM, Urban RJ, Metz DD, Rodgers BD, Blackman MR.** Comparison of GH, IGF-I, and testosterone with mRNA of receptors and myostatin in skeletal muscle in older men. *Am J Physiol Endocrinol Metab* 281: E1159–E1164, 2001.
39. **McLean E, Devlin RH, Byatt JC, Clarke WC, Donaldson EM.** Impact of a controlled release formulation of recombinant bovine growth hormone upon growth and seawater adaptation in coho (*Oncorhynchus kisutch*) and chinook (*Oncorhynchus tshawytscha*) salmon. *Aquaculture* 156: 113, 1997.
40. **McPherron AC, Lawler AM, Lee SJ.** Regulation of skeletal muscle mass in mice by a new TGF-beta superfamily member. *Nature* 387: 83–90, 1997.
41. **Mori T, Hiraka I, Kurata Y, Kawachi H, Mano N, Devlin RH, Nagoya H, Araki K.** Changes in hepatic gene expression related to innate immunity, growth and iron metabolism in GH-transgenic amago salmon (*Oncorhynchus masou*) by cDNA subtraction and microarray analysis, and serum lysozyme activity. *Gen Comp Endocrinol* 151: 42–54, 2007.
42. **Moriyama S.** Increased plasma insulin-like growth factor-I (IGF-I) following oral and intraperitoneal administration of growth hormone to rainbow trout, *Oncorhynchus mykiss*. *Growth Regul* 5: 164–167, 1995.
43. **Norbeck LA, Kittilson JD, Sheridan MA.** Resolving the growth-promoting and metabolic effects of growth hormone: differential regulation of GH-IGF-I system components. *Gen Comp Endocrinol* 151: 332–341, 2007.

44. **O'Connor PK, Reich B, Sheridan MA.** Growth hormone stimulates hepatic lipid mobilization in rainbow trout, *Oncorhynchus mykiss*. *J Comp Physiol B* 163: 427–431, 1993.
45. **Provenzano M, Mocellin S.** Complementary techniques: validation of gene expression data by quantitative real time PCR. *Adv Exp Med Biol* 593: 66–73, 2007.
46. **Purcell MK, Nichols KM, Winton JR, Kurath G, Thorgaard GH, Wheeler P, Hansen JD, Herwig RP, Park LK.** Comprehensive gene expression profiling following DNA vaccination of rainbow trout against infectious hematopoietic necrosis virus. *Mol Immunol* 43: 2089–2106, 2006.
47. **Rahman MA, Maclean N.** Growth performance of transgenic tilapia containing an exogenous piscine growth hormone gene. *Aquaculture* 173: 333–346, 1999.
48. **Rescan PY, Jutel I, Ralliere C.** Two myostatin genes are differentially expressed in myotomal muscles of the trout (*Oncorhynchus mykiss*). *J Exp Biol* 204: 3523–3529, 2001.
49. **Rise ML, Douglas SE, Sakhrani D, Williams J, Ewart KV, Rise M, Davidson WS, Koop BF, Devlin RH.** Multiple microarray platforms utilized for hepatic gene expression profiling of GH transgenic coho salmon with and without ration restriction. *J Mol Endocrinol* 37: 259–282, 2006.
50. **Roberts SB, McCauley LA, Devlin RH, Goetz FW.** Transgenic salmon overexpressing growth hormone exhibit decreased myostatin transcript and protein expression. *J Exp Biol* 207: 3741–3748, 2004.
51. **Salem M, Kenney PB, Rexroad CE 3rd, Yao J.** Microarray gene expression analysis in atrophying rainbow trout muscle: a unique non-mammalian muscle degradation model. *Physiol Genomics* 28: 33–45, 2006.
52. **Schulte PM, Down NE, Donaldson EM, Souza LM.** Experimental administration of recombinant bovine growth hormone to juvenile rainbow trout (*Salmo gairdneri*) by injection or by immersion. *Aquaculture* 76: 145, 1989.
53. **Shamblott MJ, Cheng CM, Bolt D, Chen TT.** Appearance of insulin-like growth factor mRNA in the liver and pyloric ceca of a teleost in response to exogenous growth hormone. *Proc Natl Acad Sci USA* 92: 6943–6946, 1995.
54. **Shamblott MJ, Leung S, Greene MW, Chen TT.** Characterization of a teleost insulin-like growth factor II (IGF-II) gene: evidence for promoter CCAAT/enhancer-binding protein (C/EBP) sites, and the presence of hepatic C/EBP. *Mol Mar Biol Biotechnol* 7: 181–190, 1998.
55. **Shen Y, Lindberg A, Olivecrona G.** Apolipoprotein CII from rainbow trout (*Oncorhynchus mykiss*) is functionally active but structurally very different from mammalian apolipoprotein CII. *Gene* 254: 189–198, 2000.
56. **Shepherd BS, Drennon K, Johnson J, Nichols JW, Playle RC, Singer TD, Vijayan MM.** Salinity acclimation affects the somatotropic axis in rainbow trout. *Am J Physiol Regul Integr Comp Physiol* 288: R1385–R1395, 2005.
57. **Sheridan MA.** Effects of thyroxin, cortisol, growth hormone, and prolactin on lipid metabolism of coho salmon, *Oncorhynchus kisutch*, during smoltification. *Gen Comp Endocrinol* 64: 220–238, 1986.
58. **Smyth GK.** Limma: linear models for microarray data. In: *Bioinformatics and Computational Biology Solutions Using R and Bioconductor*, edited by Gentleman R, Carey V, Dudoit S, Irizarry R and Huber W. New York: Springer, 2005, p. 397–420.
59. **Smyth GK.** Linear models and empirical bayes methods for assessing differential expression in microarray experiments. *Stat Appl Genet Mol Biol* 3: Article3, 2004.
60. **Smyth GK, Rithie M, Thorne N, Wettenhall J.** *Limma: Linear Models for Microarray Data User's Guide*. Melbourne, Australia: Walter and Eliza Hall Institute of Medical Research, 2007.
61. **Smyth GK, Speed T.** Normalization of cDNA microarray data. *Methods* 31: 265–273, 2003.
62. **Sohm F, Manfroid I, Pezet A, Rentier-Delrue F, Rand-Weaver M, Kelly PA, Boeuf G, Postel-Vinay MC, de Luze A, Edery M.** Identification and modulation of a growth hormone-binding protein in rainbow trout (*Oncorhynchus mykiss*) plasma during seawater adaptation. *Gen Comp Endocrinol* 111: 216–224, 1998.
63. **von Schalburg KR, Rise ML, Cooper GA, Brown GD, Gibbs AR, Nelson CC, Davidson WS, Koop BF.** Fish and chips: various methodologies demonstrate utility of a 16,006-gene salmonid microarray. *BMC Genomics* 6: 126, 2005.
64. **Vong QP, Chan KM, Cheng CH.** Quantification of common carp (*Cyprinus carpio*) IGF-I and IGF-II mRNA by real-time PCR: differential regulation of expression by GH. *J Endocrinol* 178: 513–521, 2003.
65. **Wang Y, Barbacioru C, Hyland F, Xiao W, Hunkapiller KL, Blake J, Chan F, Gonzalez C, Zhang L, Samaha RR.** Large scale real-time PCR validation on gene expression measurements from two commercial long-oligonucleotide microarrays. *BMC Genomics* 7: 59, 2006.
66. **Yada T, Azuma T, Takagi Y.** Stimulation of non-specific immune functions in seawater-acclimated rainbow trout, *Oncorhynchus mykiss*, with reference to the role of growth hormone. *Comp Biochem Physiol B Biochem Mol Biol* 129: 695–701, 2001.